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A Unifying Concept for the Prebiotic Formation of RNA Pyrimidine Nucleosides

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The question of how nucleosides might have formed as essential precursor molecules on the early Earth is one of the many challenges associated with the origin of life. In this context, the prebiotic synthesis of pyrimidine nucleosides is controversially discussed. For the pyrimidines, two at first glance contradictory prebiotically plausible reaction pathways have been proposed, based on either oxazole or isoxazole chemistry. This study shows that these two reaction sequences can be merged under prebiotically reasonable conditions, suggesting that both pathways could have co-existed and possibly interacted. The key precursor 3-aminoisoxazole was found to react with the key intermediate of the oxazole route (ribo-2-(methylthio)oxazoline), to form a ribo-isoxazole-oxazoline hybrid structure, which collapses upon reductive N–O bond cleavage to give the nucleoside cytidine. The data suggest that different, interacting prebiotically plausible chemical pathways may have created the key molecules of life on the early Earth.

Introduction

Life is a complex phenomenon that relies on the availability of a large number of building blocks such as amino acids, nucleosides, and molecules that can build cell walls and establish complex metabolic networks.[1] The question of how all these "molecules of life" could have formed in the absence of an efficient biosynthetic machinery at the dawn of life is one of the greatest scientific challenges.[1] The first steps towards life required the formation of higher order structures from molecules that must have formed in the abiotic environment on the early Earth.[2] These molecules then learned to perform peptide synthesis.[3] Urey and Miller, for example, simulated putative early Earth conditions and found that amino acids can form by lightning through an atmosphere composed of H_2, H_2O, CH_4, and NH_3.[4] Since then, scientists have been trying to unravel the chemical networks that could lead to the formation of the building blocks of life under prebiotically plausible conditions. In this context, special attention was and is paid to pathways that can generate amino acids,[5] establish potential early metabolic pathways,[6] or lead to the formation of purine and pyrimidine nucleosides.[7]

Particularly, the question of how the pyrimidine nucleosides could have formed under plausible early Earth conditions has been a long-standing one. Based on the chemistry proposed by Orgel and co-workers,[8] Sutherland and Powner (Figure 1) reported in seminal pieces of work that cyanamide (**1**) or thiocyanic acid (**2**) can react with glycolaldehyde (**3**) to give either 2-aminooxazole (**4**)[9] or 2-thiooxazole (**5**).[10] These precursors were found to react with glyceraldehyde (**6**) to ribo-2-aminooxazoline **7**[9] and ribo-2-thiooxazoline **8**,[10] respectively. Ribo-2-aminooxazoline **7** is proposed to have reacted directly with cyanoacetylene (**9**) to form pyrimidine nucleosides.[11] In addition, methylation of ribo-2-thiooxazoline **8** via cyanoacetylene (**9**) and methanethiol (**10**) to ribo-2-(methylthio)oxazoline **11** could have initiated the formation of 8-oxopurine nucleosides under early Earth conditions.[10] The reported pathways have the advantage of circumventing free ribose (**12**) for nucleosidation, although recent data suggest that the availability of ribose (**12**) may not be an insurmountable problem.[12] A potential hurdle of the pathways is the need for reactive cyanoacetylene (**9**) at a late stage of the synthesis, since it has a half-life of only 11 d in an aqueous environment (pH 9, 30 °C).[13]

Carell and co-workers discovered that cyanoacetylene (**9**) can be directly trapped by hydroxylamine (NH_2OH, **13**), already in the first step of the isoxazole pathway.[14] Hydroxylamine **13** forms from NO_2^- and SO_2[14] and can potentially accumulate in the absence of Fe^{2+}. In the presence of Fe^{2+} and if not directly trapped, **13** will be reduced to ammonia,[15] which is a starting material for prebiotic pathways to amino acids.[16] The reaction of **13** with **9** gives 3-aminoisoxazole (**14**), which reacts with urea and ribose (**12**) via **15** to form isoxazole ribonucleosides and

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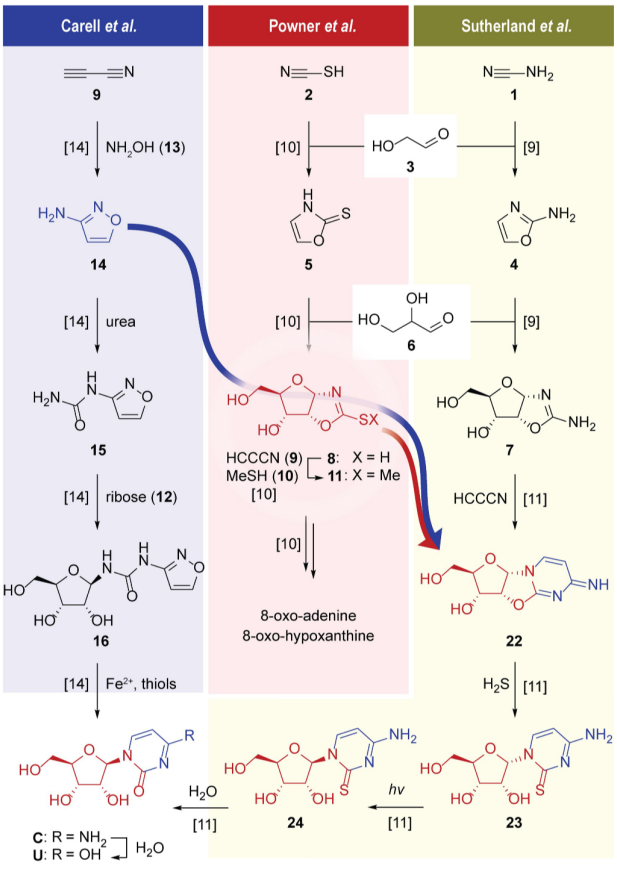


Figure 1. Depiction of the Sutherland, Powner, and Carell pathways to pyrimidines and 8-oxo-purines, respectively, together with a schematic presentation of the link between these pathways.

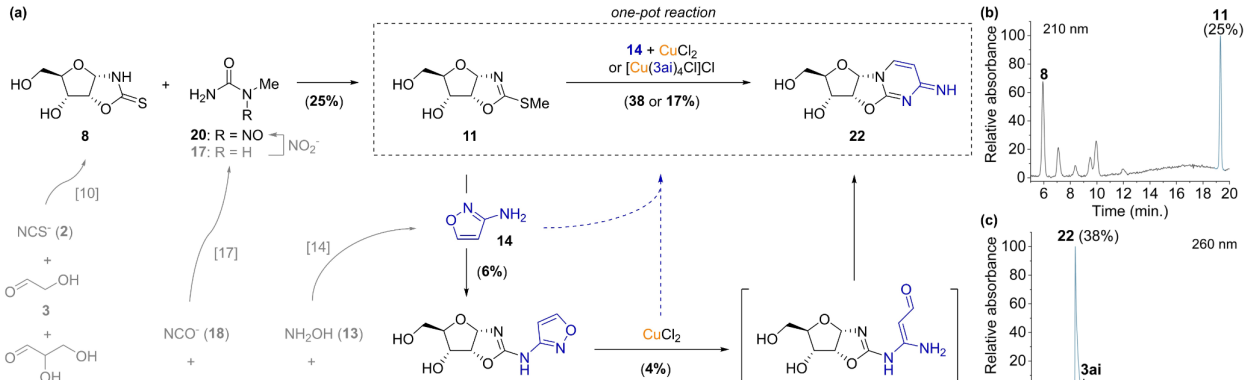
pathway avoids late-stage reaction with cyanoacetylene (**9**), it is dependent on the availability of free ribose (**12**).

Here we investigated if the two seemingly incompatible oxazole and isoxazole pathways can be combined to give pyrimidine nucleosides without the need for ribose and late-stage addition of cyanoacetylene (**9**).

Results and Discussion

To establish a combined pathway, we recapitulated that thiocyanate (**2**) and glycolaldehyde (**3**) form 2-thiooxazole (**5**), which reacts with glyceraldehyde (**6**) to provide ribo-2-thiooxazoline **8**.[10] Deviating from the originally reported methylation step, we found that methylation is possible starting with *N*-methylurea (**17**), which forms from cyanate (**18**) and methylamine (**19**) (Figure 2a).[17] Nitrosation of **17** (via NO_2^-) generates *N*-methyl-*N*-nitrosourea (**20**), which decomposes spontaneously under basic conditions to diazomethane,[17] allowing selective methylation of the thiol functionality to give **11** in 25% yield (Figure 2b).

At the beginning of our reaction sequence, cyanoacetylene (**9**) reacts with hydroxylamine (**13**) to give 3-aminoisoxazole (**14**), which forms even in the presence of Fe^{2+} as shown in this study. We have now discovered that **14** reacts with ribo-2-(methylthio)-oxazoline **11** under formation of product **21**. Although we did not optimize the reaction, we observed the formation of ribo-*N*-isoxazolyl-2-aminooxazoline **21** in about 6% yield, next to remaining starting material. Subsequent cleavage of the isoxazole N–O bond in the presence of Cu^{2+} and thiols then generated the anhydronucleoside **22**, which leads to cytidine and uridine along known pathways via **23** and **24**.[11] To study one-pot conditions, which are prebiotically more plausible, we mixed ribo-2-(methylthio)oxazoline **11** with 3-aminoisoxazole (**14**) and $CuCl_2$. Interestingly, we observed direct formation of the anhydro nucleoside in 38% yield under slightly acidic conditions (pH ≈ 4). The chromatogram depicted in Figure 2c shows the efficient conversion of the Powner intermediate with the Carell isoxazole intermediate to the joined structure **22**. In this scenario we believe that the MeSH



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leaving group itself provides the reducing power and converts Cu^{2+} to Cu^{+}, which subsequently catalyzes the N–O bond opening.[18] When we instead performed the reaction with Fe^{2+}, which was highly abundant on the early Earth,[19] **22** formed only in traces (<1%). In the presence of both Cu^{2+} and Fe^{2+}, formation of **22** was also observed but with reduced yields, indicating that Fe^{2+} hampers the reaction. Other metal ions such as Ca^{2+}, Co^{2+}, Ni^{2+} or Zn^{2+}, which are considered to be prebiotically available,[19] allowed the formation of **22** at best in traces. In the absence of metal salts, the one-pot formation of **22** does not occur.

The new combined pathway reported here avoids the need for free ribose and the late-stage reaction of cyanoacetylene. However, a potential problem could be the reaction of ribo-2-(methylthio)oxazoline **11** with other nucleophiles in the absence of a 3-aminoisoxazole (**14**), which would waste starting materials. In additions, the formation of 3-aminoisoxazole (**14**) requires cyanoacetylene (**9**), and if **9** was only formed in traces, this would also apply to 3-aminoisoxazole (**14**). A potential solution to this issue could be an enrichment process that allows formation of 3-aminoisoxazole (**14**) deposits. We therefore investigated its interaction with the divalent metal ions Ca^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}. We found that 3-aminoisoxazole (**14**) forms stable complexes in the presence of each metal ion, which crystallize from solution (Figure 3). This broad possibility for the deposition and accumulation of 3-aminoisoxazole (**14**) supports the linked pathway model proposed here. Next, we performed an experiment mixing all the above-mentioned metal salts in the presence of 3-aminoisoxazole (**14**), and to our delight, we observed that the Cu^{2+} complex ($[Cu(3ai)_4Cl]Cl$) crystallizes first. Since Cu^{2+} is considered a prebiotically less abundant metal ion and its exact concentrations are questionable,[19] it is remarkable that 3-aminoisoxazole (**14**) allows Cu^{2+} enrichment. This observation further supports the plausibility of the chemistry reported here, as Cu^{+} is needed to catalyze the N–O bond opening and initiates the reaction cascade to cytidine (Figure 2). To demonstrate that the enriched complex ($[Cu(3ai)_4Cl]Cl$) can be used directly for the one-pot reaction, we mixed the isolated crystals with ribo-2-(methylthio)oxazoline **11** under slightly acidic conditions (pH≈4). After one day at 25 °C, anhydronucleoside **22** was indeed obtained in 17% yield, showing that both the organic and inorganic parts of the deposit are chemically available.

Conclusion

The currently proposed prebiotically plausible pathways to pyrimidine nucleosides depend on oxazole[9–11] or isoxazole[14] intermediates, some of which form in one-pot reactions driven by wet-dry cycles. If we assume that prebiotic chemistry took place in warm, shallow ponds, as already assumed by Charles Darwin,[20] early chemical transformations on Earth were probably largely unsynchronized, so that all starting molecules and all intermediates were present in a reaction environment at the same time. In such a scenario, we must assume that multiple reactions occurred between the individual pathways. Such a scenario can be partially circumvented by extending the concept to interconnected shallow ponds, in which different chemical processes are spatially and temporally separated and may mix at certain times due to flooding or geological activities. In such a scenario, it is not unrealistic that either ribose or cyanoacetylene formed in a separate pond and later entered the main reaction site. However, even in multiple pond scenarios we must assume that different reaction pathways occurred simultaneously.

Here we show that the two pathways that have been proposed for the formation of pyrimidine nucleosides under plausible early Earth conditions, which have both specific advantages and disadvantages, can be merged to give a unified pathway. The result shows that nucleosides as privileged prebiotic molecules can form under a variety of conditions. There are many pathways to nucleosides, and they appear to be interconnected.

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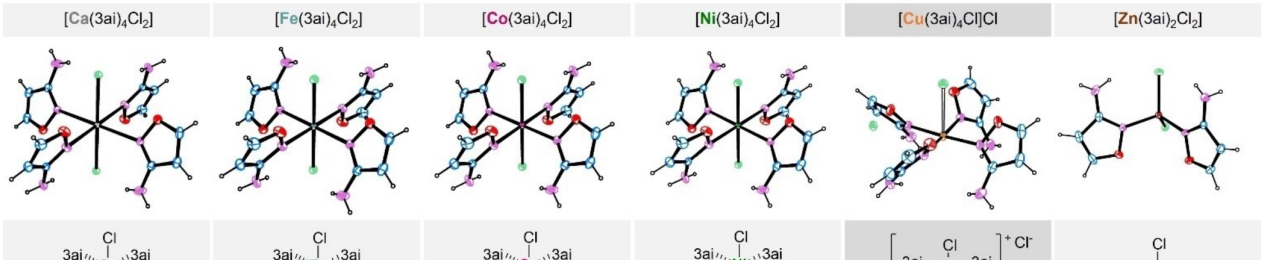

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: 3-aminoisoxazole · copper catalysis · origin of life · prebiotic chemistry · pyrimidine nucleosides

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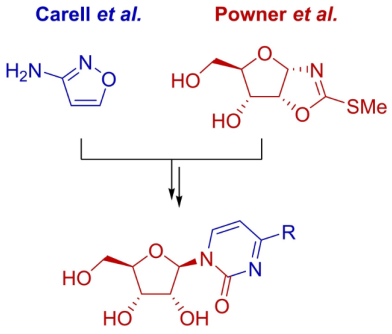
The plausible prebiotic syntheses of RNA pyrimidine nucleosides established by Carell *et al.*, Powner *et al.*, and Sutherland *et al.* were combined into a unified concept. The resulting route allows pyrimidine nucleoside formation without the need for free ribose or the late-stage addition of reactive cyanoacetylene.



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